Science of the Total Environment 626 (2018) 851–859

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Beneficial effects of restoration practices can be thwarted by climate extremes

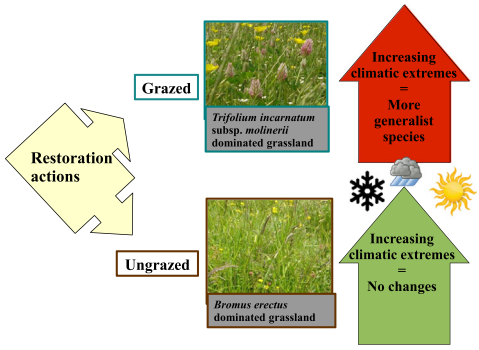
Simona Maccherini [a,*], Giovanni Bacaro [b], Michela Marignani [c]

[a] Department of Life Sciences, University of Siena, Siena, Italy
[b] Department of Life Sciences, University of Trieste, Trieste, Italy
[c] Department of Life and Environmental Sciences, Botany Division, University of Cagliari, Cagliari, Italy

HIGHLIGHTS

- The interaction of climate extremes and grazing thwarted restoration success.
- Synanthropic species took advantage, in grazed site, of the increasing climate extremes.
- Endemic species decreased with an increase of heavy rainfall events.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 October 2017
Received in revised form 12 January 2018
Accepted 12 January 2018
Available online 19 February 2018

Editor: Elena Paoletti

Keywords:
Calcareous grassland
Climate change
Grazing
Long-term ecological research
Restoration experiment
Mediterranean climate
Monitoring restoration

ABSTRACT

The impacts of climate extremes on species, communities and ecosystems have become critical concerns to science and society. Under a changing climate, how restoration outcomes are affected by extreme climate variables is a largely unknown topic. We analyzed the effects of experimental factors (grazing and sowing of native species), extreme climate events (intense precipitation and extreme temperatures indexes) and their combination on the restoration progress of a dry, calcareous grassland in Tuscany (Italy) with a 1 year before/15 years continuous annual monitoring after, control/impact (BACI) experiment. Grazing had a beneficial effect on the diversity of the grassland, while sowing had a limited impact. The climatic index that most affected the entire plant community composition was the number of very heavy precipitation days. The interaction of grazing and extreme climatic indexes had a significant detrimental effect on restoration outcomes, increasing the cover of synanthropic and Cosmopolitan-Subcosmopolitan generalist species and decreasing the cover of more valuable species such endemic species. In the richest grazed plots, species richness showed a lower sensitivity to the average precipitation per wet day but in grazed site, restoration outcomes can be negatively influenced by the intensification of precipitation and temperature extremes. In a context of progressive tropicalization of the Mediterranean area, to assist managers setting achievable restoration goals, restoration practitioners should consider that climate extremes might interfere with the beneficial effects of restoration practices.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

The increase in the frequency and magnitude of climate extremes is a well-accepted feature of forecast alterations to the global climate

* Corresponding author.
E-mail address: simona.maccherini@unisi.it (S. Maccherini).

https://doi.org/10.1016/j.scitotenv.2018.01.117
0048-9697/© 2018 Elsevier B.V. All rights reserved.

(IPCC, 2007). How climate extremes affect species, communities and ecosystems has become critical to science and society (Jentsch et al., 2011; Smith, 2011; Lloret et al., 2012; Hoover et al., 2014; Chelli et al., 2017; De Boeck et al., 2017) but several effects on biodiversity, as the implications for ecological restoration, are still unknown (Harris et al., 2006). For example, it is acknowledged that restoration outcomes can be driven by biotic and abiotic factors (Stuble et al., 2017).We know that the effect of rainfall on species cover in serpentine grassland depends on the rate of disturbance, and that extreme climate events, such as *el Niño* cycles, contribute to the invasion of non-native species (Hobbs et al., 2007). We also learned that grazing and drought interact, to determine resistance to invasion by perennial grass communities (Han and Young, 2014) and that mowing and drought have different effects on diversity of xeric and mesic communities (Maalouf et al., 2012). Nevertheless, how climate variables, such as extreme temperatures or heavy rain, interact with the progress of restoration practices is a largely unexplored topic.

The evaluation of restoration success is often undermined by the lack of clearly defined restoration goals, the scarcity of shared methods for measuring restoration efficacy and by a chronic absence of monitoring, as demonstrated for grasslands (Waldén and Lindborg, 2016). The methodological shortcomings in monitoring are accompanied by the tendency of ecologists to study vegetation change and attribute it to individual causes when, instead, the study of multiple causes and effects and their interactions is essential to understanding, managing and restoring ecosystems more effectively (Hobbs et al., 2007; Stuble et al., 2017). In this context, long-term data become increasingly important to interpreting patterns of response on entire community composition or species distributions and to measure the long-term restoration success (Hobbs et al., 2007).

Grasslands are particularly important for the diversity of small-scale vascular plants (Butaye et al., 2005; Dengler et al., 2014) since semi-natural, temperate grasslands host the world record on the number of taxa in $1m^2$ (Wilson et al., 2012). The conservation of calcareous grassland communities is strongly advocated for, due to their capacity to support high biodiversity as well as rare and/or endangered species (Willems, 2001). However, changes in land use have resulted in a substantial decline in the extent and ecological quality of semi-natural grassland communities, over the past two decades across Europe (van Dijk, 1991; Bakker and Berendse, 1999; Critchley et al., 2004; Ridding et al., 2015). Restoration activities have been undertaken in many sites to improve the habitat conditions and increase the area to prevent any further losses of their ecological services and value (Waldén and Lindborg, 2016). Maccherini and Santi (2012) using a long-term (10 years), Before/After-Control/Impact experiment (BACI) showed that different restoration practices substantially affect the structure and composition of a calcareous grassland. After 10 years of observation, the perennial grass *Bromus erectus* and the annual legume *Trifolium incarnatum* subsp. *molinerii* were the species most influenced by restoration measures: grazed plots were dominated by *T. incarnatum* subsp. *molinerii,* an annual Euri-Mediterranean species typical of ex-arable and arable land, while the perennial *B. erectus,* a Paleotemperate species typical of grasslands, dominated ungrazed plots (Maccherini and Santi, 2012).

The Mediterranean Basin is considered a climate change hotspot sensitive to global warming (Giorgi and Lionello, 2008). In Italy, during the last 55 years, extreme temperature indices showed a general warming trend for the entire peninsula (Toreti and Desiato, 2008; Chelli et al., 2017) and in future decades heavy rainfall and summer heat waves are expected (ISAC-CNR, 2009). In Tuscany, precipitation indices showed a negative trend (years 1955–2010) in the number of wet days, associated with an increase in the contribution of heavy rainfall events to total precipitation (Regione Toscana, 2012).

Under a climate change scenario, characterized by an intensification of extreme climate episodes, after 5 years from the study focusing on restoration strategies (Maccherini and Santi, 2012), we used a *1 year before - 15 years after continuous annual* control-impact (Before/After-Control/Impact) experiment to investigate how experimental factors and extreme climate events affect the restoration of a dry, calcareous grassland. Specifically, we tested the following questions: do climatic extremes interact with experimental factors (grazing and sowing)? If so, how do they interact? Which group of species is more resistant to the influence of experimental factors and/or climatic extremes?

2. Materials and methods

2.1. Study area

The experiment was carried out in close proximity to the summit of Monte Labbro (42°49′13″N, 11°31′33″E), Tuscany (Italy), a predominantly calcareous massif on the Uccellina-Monte Amiata ridge (Lazzarotto, 1993). The area is included in a Site of Community Importance/Special Protected Area (SCI/SPA, "Monte Labbro and Upper Albegna Valley"). The summit area (1193 m a.s.l.) has been grazed for centuries, mostly by sheep, with human traces dating back to the Bronze Age (Pistoi, 1989). From the 1960s onwards, the grazing pressure decreased, triggering the secondary succession of semi-natural grasslands into increasingly dense scrublands. The area is characterized by a mosaic of community types, including well preserved, semi-natural, dry, calcareous grasslands with few or no shrubs, overgrown grasslands, dense scrublands with *Prunus spinosa*, *Rubus ulmifolius* and *Cytisus scoparius*, and woodlands with *Acer campestre* and *Acer monspessulanum*. In the period comprising late summer of 2000 to early spring 2001, the laborers manually cut all shrubs (except all individuals of *Juniperus communis* and some of *P. spinosa, Rosa canina* and *Crataegus monogyna*, for faunistic conservation purposes; for more details see Maccherini et al., 2007, 2014; Maccherini and Santi, 2012).

We located the experimental plots in a cleared overgrown pasture (before cutting, *Prunus spinosa* covered 80%), grazed by donkeys, which were reintroduced into the area few years before restoration management; the site is occasionally grazed by sheep, hares and cattle. Stock density ($0.5\ ha^{-1}\ yr^{-1}$) did not change during the years of observation.

2.2. Experimental design, measurements and climate data

We conducted a 1 year before/15 years after-control/impact experiment (BACI), with continuous monitoring because this design overcomes the problem of attributing changes to an impact rather than natural variability (Stewart-Oaten et al., 1986). In fact, to conclude that the observed changes in restored site (Impact site) were determined by restoration actions (i.e. experimental factors), it's necessary to compare the plant composition of the restored sites with degraded areas, similar to the restored ones, which were not manipulated or managed (Control site). This comparison allows us to take into account and measure the natural variability among sites in the absence of disturbance (Chapman and Underwood, 2000; Benedetti-Cecchi and Osio, 2007). In this context, the restoration measures (i.e. grazing and sowing of native species, the experimental factors) are the source of disturbance and the effects of the restoration are defined as the differences in community composition between the two levels of factor grazing and sowing.

We tested the effects of grazing (two levels: grazed and ungrazed) and the sowing of native species (two levels: sown, non-sown) on restoration of a calcareous grassland communities. We established, in spring 2001, a randomized, block design in an area with a dense cover of *P. spinosa* (80%) that was cut in the early spring of 2001. The area lies on a moderately steep slope (15°) with a south-west aspect. Four 3×5 m experimental plots were established in each of four blocks stratified on elevation. In the summer of 2001, we harvested seeds from a reference grassland community, in the study area (Maccherini et al., 2007). Individual plots in each block were randomly assigned to one

of four treatments: no grazing or sowing with native species; sowing without grazing; grazing without sowing; sowing and grazing. The treatments were carried out on 4th October 2001. We used a sowing rate of 4 g m^{-2}, which has been considered adequate to recreate calcareous grassland (see Stevenson et al., 1995). Livestock were excluded by fencing off the respective plots.

Vegetation surveys were made annually in the sixteen 1 × 2 m subplots located at the center of each experimental plot, in late June to early July, starting in 2001 (before treatment). No data were recorded in 2005. All vascular plant species, growing in each plot, were recorded and their cover was estimated using a point quadrant method (Moore and Chapman, 1986), with a density of 100 pins m^{-2}. If more than one species was touched as the pin was lowered, more than one hit was recorded, so that total cover of species could exceed 100%. Species present in a plot but not touched by any pins were recorded with an arbitrary cover of 0.05%. Previous results showed that managed plots were very similar to the control plots in 2001, indicating a similar community composition in the year preceding the exclusion of livestock and the sowing of seeds of native species. The dissimilarity between grazed and sown and grazed plots increased progressively in 2002 and 2003. Subsequently, these dissimilarities remained relatively stable, suggesting small effect of sowing compared to grazing (Maccherini and Santi, 2012).

The species were divided into groups to investigate the effect of the climatic extremes and the treatment not only on the entire plant community, but to explore the effects exerted on the different portion of the community separated, accordingly to an ecological perspective. In particular, plant species were classified by their habitat and chorological type according to Pignatti (1982). In group H1 we gathered the species defined as exclusive of grassland, pasture, rocky slope and rock (41 species) characteristic of the reference grassland community, with guiding species such as *Sedum album*, *Sanguisorba minor, Thymus longicaulis* and the endemics to the Italian territory *Centaurea deusta* and *Phleum ambiguum* (Maccherini et al., 2007). The group H2 is composed by a total of 46 species, living in grassland but also in other habitat such as *Tordylium maximum, Dactylis hispanica* and *Veronica arvensis*. Species of scrubland and woodland, like the endemic *Myosotis decumbens* subsp. *florentina*, or shrubs like *C. monogyna* and *P. spinosa* were included in the group H3 (11 species). All other generalist species of human habitat, such as the weed species *Capsella bursa-pastoris* and *Plantago lanceolata*, were grouped together in H4 (synanthrope species, Di Castri et al., 1990). For chrological categories we defined a group of species endemic to the Italian territory or part of this (E, 7 species); a group of species restricted to the Mediterranean coasts (M, 10 species); a group of European, Boreal and Temperate species (EBT, 85 species) and a contingent of Cosmopolitan and Subcosmopolitan species (C, 27 species).

We calculated the sum of the percentage cover for all species belonging to each habitat or chorological type for each plot and year of observation. This classification allows us to understand which groups of species are more resistant to climate change: we identify, as the most valuable, the species exclusive of grasslands (H1, guiding species for the target grassland) and the endemics, species restricted to a defined geographical area that, if they respond negatively to climate change, would be highly threatened. We can oppose those valuable species to the weeds (synanthropic species) and the widespread species (Cosmopolitan and Subcosmopolitan) that, albeit influenced negatively by change climate, would in any case be, in absolute terms, less threatened in terms of conservation, because of their wide distribution.

To analyze how climate extremes affect species and plant communities subjected to the restoration project, we adopted a selection of the extreme temperature and precipitation indices, recommended by the Expert Team on Climate Change Detection and Indices (ETCCDI) for monitoring changes in climatic extremes (Zhang et al., 2011). The 'ETCCDI' indices (Frich et al., 2002) are a suite of indices, based on the European Climate Assessment (ECA), developed to analyze trends for the second half of the 20th century (Zhang et al., 2011). Climatic data were obtained from the closest climatic station (S. Fiora, 687 m a.s.l, 5.56 km from the study area). Whenever missing values were encountered, data were substituted from Roccalbegna or Castel del Piano station; all climatic stations were <7.5 km from the study area. For the period 2000–2016 we calculated: Frost days (FD)- number of days with $T_{min} \leq 0$; Summer days (SU30) - number of days with $T_{max} > 30$; number of extremely heavy precipitation days (R20) - number of days with precipitation ≥ 20 mm/day and Simple daily intensity index (SDII) - the ratio of total precipitation to the number of wet days (with precipitation ≥1 mm, unit mm/day). Extreme values were those used to analyze climate changes for Tuscany (Regione Toscana, 2012).

2.3. Statistical analysis

Spearman's rank correlation was performed to determine any significant correlation between climatic extremes and year of observation. We performed multivariate and univariate permutational analysis of covariance (Anderson, 2001) using as the response variables: community composition (presence of all species with their percentage cover for all treatments and years of observation), habitat type, chorological type, number of species and the percentage cover of two species that responded most to grazing: *T. incarnatum subsp. molinerii* and *B. erectus* (Maccherini and Santi, 2012). The advantage of the permutation approach is that the resulting test is "distribution free" and not constrained by many of the typical assumptions of parametric statistics.

We analyzed the effects of periods of observation - BA (fixed, two levels: Before - one year before, and After – all 15 years after, restoration management), grazing - G (fixed, two levels: grazed and ungrazed), sowing - S (fixed two levels sown and not sown), Block (random, four levels) and year of observation - Year (random, 15 levels nested within BA) and used the four climatic extremes as covariates. We used for SU30 the sum of the period ranging from June of the previous year to May of the year of sampling, assuming this was the most influential period on the growing season (i.e., September of the previous year-June of the year of sampling). For FD, R20 and SDII we used the sum of the period ranging from December of the previous year to May of the year of sampling. These time spans represent the periods of the year more affecting vegetation in sites similar for temperature and precipitation data (see Chiarucci and Maccherini, 2007).

We also investigated the fixed effects of the interactions: BA × G, BA × S, which - if significant - implies a long-lasting effect of restoration observable 15 years after restoration application; covariates × G and covariates × S which - if significant - indicates a different effect of the covariate between the two levels of factor grazing and sowing. All tests were performed with 999 permutations of residuals under a reduced model (Anderson and ter Braak, 2003). Multivariate analyses were done on the basis of a Bray–Curtis dissimilarity matrix calculated from $ln\,(x + 1)$-transformed percentage cover data, univariate analyses were done on the basis of a Euclidean distance matrix calculated from untransformed data. Significant terms relevant to the hypotheses were investigated through post hoc pair-wise comparisons using PERMANOVA *t*-test and 999 permutations. Analyses were performed using the PERMANOVA routine in the PRIMER v6 computer program (Clarke and Gorley, 2006), including the add-on package PERMANOVA + (Anderson et al., 2008).

When interaction covariate × G or covariate × S was significant, we performed the Pearson correlation test to determine any significant linear correlations between the climatic extremes index and dependent variables separately for the two levels of factor grazing or sowing. We used descriptive graphs to show the effect of climatic extremes on some parameters. Correlation analyses were performed using STATISTICA for Windows 6.0 (StatSoft Italia srl, 2001). We set alpha to 0.05.

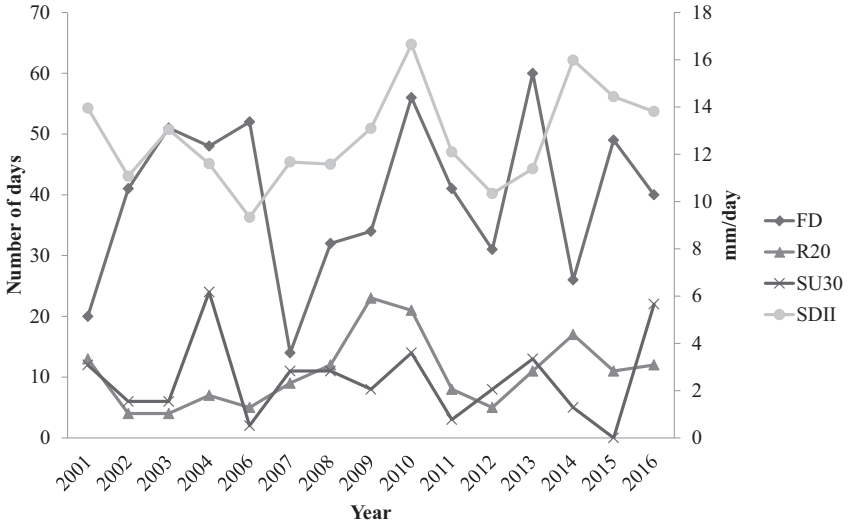


Fig. 1. Frost days (FD), Summer days (SU30), very heavy precipitation days (R20) and Simple daily intensity index (SDII) over 15 years of observation. Climatic data are presented by autumn, summer and spring before data collection for SU30 and winter and spring before data collection for FD, R20 and SDII.

3. Results

Climatic extremes data that were calculated for the period of interest were quite variable (Fig. 1). Correlation analyses showed a significant positive relationship between year of observation and precipitation extreme (R20: rho = 0.37, P < .05; SDII: rho = 0.3, P < .05) while temperature data did not show significant correlation (FD: rho = 0.08, SU30: rho = −0.07).

Results of permutational analysis of covariance for the entire community composition, the number of species, *T. incarnatum* subsp. *molinerii* and *B. erectus* showed that: the year of observation had a significant effect for community composition, the number of species and *B. erectus* cover; interaction BA × G was significant for all the response variables (Table 1). Block was significant for all the response variable except for the number of species (Table 1). For community composition, R20 showed a significant effect as well as the interactions SDII × G and R20 × G. The interaction SDII × G was significant also for the number of species. In ungrazed plot the number of species showed a negative significant correlation with SDII (r = −0. 24, P < .05) (Table 1; Fig. 2). *T. incarnatum* subsp. *molinerii* showed a higher sensitivity to climate with respect to *B. erectus* being positively correlated with SU30 in grazed plot (r = 0.30, P < .01) (Table 1; Fig. 3).

The results of permutational analysis of covariance, for different chorological and habitat types, showed that the year of observation had a significant effect on percentage cover for all dependent variables excluding C species (Table 2). Interaction BA × G was significant only for H4 and C species while BA × S was never significant indicating no effect of sowing in restoration. Number of species significantly increased

Table 1
Results of permutational analysis of covariance for entire community composition (multivariate), number of species, percentage cover of *T. incarnatum* subsp. *molinerii* and *B. erectus* in grassland plots.

Source of variation		Multivariate community composition		Number of species		*T. incarnatum* subsp. *molinerii*		*B. erectus*	
	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Frost days (*FD*)	1	3387.1	0.56	148.71	0.2	82.54	0.15	286.18	0.56
Summer days (*SU30*)	1	4293.7	0.77	27.18	0.04	424.05	0.8	1427.2	2.91
Number of very heavy precipitation days (*R20*)	1	15,255	2.48***	110.34	0.15	12.22	0.03	265.34	0.52
Simple daily intensity index (*SDII*)	1	6219	1.01	373.29	0.5	170.37	0.29	384.49	0.74
Period (*BA*)	1	7360.5	1.2	148.72	0.2	271.38	0.47	1365	2.56
Sowing (*S*)	1	7037.5	6.52***	37.604	1.64	14.43	0.42	19.72	0.13
Grazing (*G*)	1	50,758	47.52***	2489.7	116.18***	12,750	388.68***	15,031	100.01***
Block	3	6618.4	5.56***	0.73	0.03	121.3	3.5*	1380.8	9.14***
Year (*BA*)	10	5586.9	5.25***	674.23	31.45***	534.72	16.32	491.28	3.28**
FD × *S*	1	524.8	0.49	38.5	1.8	1.352	0.04	31.15	0.21
FD × *G*	1	1428.5	1.34	33.48	1.56	301.96	9.22**	225.64	1.51
SU30 × *S*	1	285.88	0.27	8.36	0.39	16.66	0.51	87.1	0.58
SU30 × *G*	1	1660.1	1.56	2.16	0.1	801.88	24.47***	0.01	0
R20 × *S*	1	1111.6	1.04	43.57	2.03	123.03	3.75*	53.1	0.35
R20 × *G*	1	2996.9	2.81***	25.6	1.19	207.19	6.32*	351.62	2.35
SDII × *S*	1	730.44	0.69	50.7	2.36	7.909	0.24	79.64	0.53
SDII × *G*	1	2474.4	2.3232***	82.4	3.84*	255.91	7.81**	55.75	0.37
BA × *S*	1	872.14	0.82	6.54	0.31	34.434	1.05	12.94	0.09
BA × *G*	1	5025.4	4.72***	335.75	15.66***	814.41	24.85***	891.27	5.95*
Residual	209	1065.1		21.43	0.2	32.767		149.78	
Total	239			148.71	0.04				

* $P \leq .05$.
** $P \leq .01$.
*** $P \leq .001$.

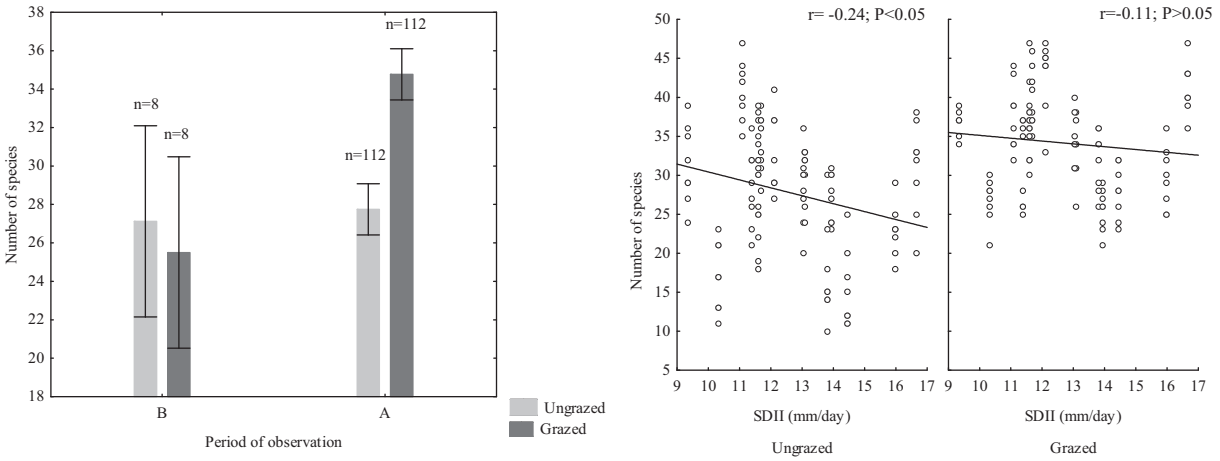


Fig. 2. Number of species for the two periods of observation: before (B) and after (A) restoration management (on the left) and linear correlations between Simple daily intensity index (SDII) and number of species separately for the two levels of factor grazing (on the right). Bars represents standard error.

only in grazed plots after management ($t = 1.81$, $P = .028$) and in the period after management, was higher in the grazed plots than in the ungrazed ones ($t = 3.024$, $P = .004$) (Fig. 2). Percentage cover of *T. incarnatum* subsp. *molinerii* in the period after management, was higher in the grazed plots than in the ungrazed ones ($t = 20.227$, $P = .001$) (Fig. 2). Block was significant for all the dependent variables less than H2 and EBT species (Table 2). Covariates had a non-significant effect on the percentage species of the four habitat types but for the H4 species the interactions FD × G, SU30 × G and R20 × G were significant, revealing a different effect of the covariates between the two levels of factor grazing (Table 2).

Only percentages of C species, independent of experimental factors, were significantly negatively affected by FD and SDII and positively by R20; R20 and FD however showed a significant interaction with factor G (Table 2, Fig. 4). A significant interaction R20 × G was shown by endemic (E) species while the two levels of factor G were differently influenced by FD (Table 2, Fig. 4). Significant relationships between extreme climate indices and species type were shown only in grazed plots. An increase of SU30 and R20 provoked an increase of H4 species ($r = 0.26$, $P < .05$ and r 0.37, $P < .05$ respectively). An increase of R20 positively affected cover of C ($r = 0.32$, $P < .05$) and negatively that of E species ($r = -0.30$, $P < .05$); a decrease in FD provoked a significant decrease of C species ($r = 0.25$, $P < .05$). All the other relationships were not statistically significant.

4. Discussion

After 16 years of observation, restoration sites showed a persistent dominant response pattern to experimental factors: factor sowing did not have a significant effect on response variables; grazing, on the other hand, affected the abundance of Cosmopolitan-Subcosmopolitan and synanthropic species. The results of this long-term study confirmed that the entire community composition was significantly affected by climate, in particular, by extreme precipitation days: it is known that the structure and the diversity of grasslands depend on climatic trends, such as inter-annual or multi-annual fluctuations of precipitation or temperature, and on the interaction of temperature and rainfall (see Dzwonko and Loster, 2011). Our results are in line with other studies who found that water-related climate changes exert dramatic impacts on the primary productivity, species composition, trophic relationships, diversity and ecosystem functioning of natural communities, especially in arid and semiarid ecosystems (Eskelinen and Harrison, 2015). In the same way, grassland restoration is influenced by moisture regime (Galvánek and Leps, 2009), annual temperature and total precipitation (Stuble et al., 2017) or rainfall manipulation (Suttle and Thomsen, 2007).

We observed that extreme climatic events interact with experimental factors, changing the restoration outcomes: climatic variables influenced community characteristics more in disturbed sites than in

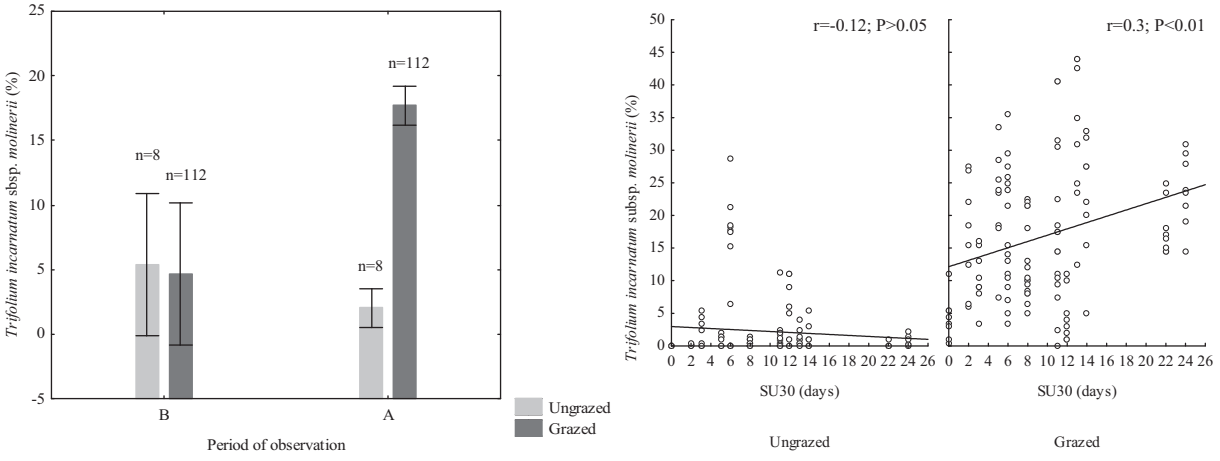


Fig. 3. Percentage cover of *T. incarnatum* subsp. *molinerii* for the two periods of observation: before (B) and after (A) restoration management (on the left) and linear correlations between number of summer days (SU30) and cover of *T. incarnatum* subsp. *molinerii* separately for the two levels of factor grazing (on the right). Bars represent standard error.

unmanaged ones, as evidenced by Chiarucci and Maccherini (2007) and Han and Young (2014). In grazed plots, synanthropic, together with endemic and Cosmopolitan-Subcosmopolitan species, were the least resistant to climate extremes, while species typical of grasslands, scrublands and woodlands showed high resistance, in the manner of Mediterranean, European, Boreal and Temperate species. Grazing disturbance created the conditions for synanthropic species to invade, through increasing summer temperature and extremely heavy precipitation days. Similar results were found by Han and Young (2014) in perennial grassland communities, where an invasive species was facilitated by grazing, combined with drought conditions. These results seem to be in contrast with the finding of Hobbs et al. (2007) where a more significant correlation of species with rainfall occurs in the enclosures and not in grazed plots. Dominant species, in serpentine grasslands, seem to be more affected by variation in rainfall than species with intermediate or low abundances, that respond more to the experimental manipulation (Hobbs et al., 2007). In our study the two dominant species, more sensitive to grazing, showed an opposite pattern: *B. erectus* showed a high resistance to climate extremes while *T. incarnatum* subsp. *molinerii*, in grazed plots, was positively correlated with the number of days with high temperature (SU30). Similarly to widely distributed species, *T. incarnatum* subsp. *molinerii* is favored by grazing and increasing temperature: this species can be classified as a "winner" (sensu McKinney and Lockwood, 1999), an example of native "alien" or native "invasive" species (Valéry et al., 2009; Helm et al., 2015), also known as weed, opportunistic species or widespread generalist species, that normally seldom occurs in the calcareous grassland habitat. Cosmopolitan-Subcosmopolitan species presence, in disturbed plots, benefit from the increase in the number of heavy precipitation days, while those species slightly decreased with the increasing number of frost days.

In this framework, decreasing species (losers) can be exemplified by the endemic *Cerastium arvense subsp. arvense* var. *etruscum* (H3) which constantly reduced over the years (Maccherini and Santi, 2012). In our results, endemic species showed a greater sensitivity to rainfall extremes in grazed plots, decreasing their cover with an increase in the number of very heavy precipitation days; however, it is worth to

Table 2
Results of permutational analysis of covariance for percentage cover of habitat and chorological types in grassland plots.

Source of variation		H1		H2		H3		H4	
	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Frost days (*FD*)	1	394.23	0.76	30.45	0.06	875.36	2.36	21.808	0.02
Summer days (*SU30*)	1	1475.9	3	912.73	1.46	737.19	2.15	892.94	0.59
Number of very heavy precipitation days (*R20*)	1	140.54	0.3	546.08	0.8	922.27	2.48	2623	1.56
Simple daily intensity index (*SDII*)	1	139.3	0.3	6.83	0.03	572.95	1.55	2118.1	1.26
Period (*BA*)	1	758.23	1.44	4.84	0.02	853.88	2.31	798.62	0.48
Sowing (*S*)	1	393	1.93	4.08	0.03	42.74	0.24	430.41	6.55*
Grazing (*G*)	1	12,46	61.3***	1775.6	15.15***	8006.7	45.29***	20,135	322.81***
Block	3	1303.1	6.39**	290.64	2.44	759.28	4.29**	519.99	7.67***
Year (*BA*)	10	492.55	2.43*	630.17	5.38***	344.39	1.95*	1527.5	24.57***
FD × *S*	1	52.73	0.26	8.26	0.07	5.06	0.03	73.28	1.18
FD × *G*	1	436.47	2.15	323.11	2.76	135.52	0.77	257.2	4.14*
SU30 × *S*	1	201.95	1	0.1	0	2.81	0.02	14.49	0.23
SU30 × *G*	1	16.72	0.08	331.26	2.83	108.17	0.61	1012.1	16.28***
R20 × *S*	1	119.04	0.59	10.36	0.09	49.4	0.28	19.48	0.31
R20 × *G*	1	613.13	3.02	51.71	0.44	43.38	0.25	1154.1	18.56***
SDII × *S*	1	63.15	0.31	238.28	2.03	71	0.4	8.78	0.14
SDII × *G*	1	44.92	0.22	58.29	0.5	147.21	0.83	33.83	0.54
BA × *S*	1	97.43	0.48	120.12	1.03	32.49	0.18	0.17	0
BA × *G*	1	636.23	3.14	0.67	0.01	405.76	2.3	1278.8	20.57***
Residual	209	202.82		117.12		176.54		62.18	
Total	239								
		E		M		EBT		C	
Frost days (*FD*)	1	71.72	1.69	11.78	0.08	2666.40	2.63	224.21	4.76*
Summer days (*SU30*)	1	0.62	0.02	5.73	0.04	35.38	0.04	18.39	0.42
Number of very heavy precipitation days (*R20*)	1	6.05	0.17	27.95	0.18	1414.40	1.4	474.68	10.01**
Simple daily intensity index (*SDII*)	1	53.43	1.26	421.88	2.56	155.06	0.16	1106.3	23.09***
Period (*BA*)	1	27.63	0.67	0.95	0.02	4290.30	4.25	291.37	6.2*
Sowing (*S*)	1	0.11	0.01	49.10	2.29	1557.90	11.99***	1000.6	30.08***
Grazing (*G*)	1	355.75	32.12***	844.69	40***	33.28	0.26	1404.3	42.25***
Block	3	51.14	4.58**	59.50	2.75*	146.46	1.12	306.02	9.23***
Year (*BA*)	10	39.05	3.53**	149.32	7.07*	924.76	7.22***	43.38	1.31
FD × *S*	1	2.57	0.23	24.11	1.14	35.59	0.28	24.24	0.73
FD × *G*	1	16.57	1.5	103.87	4.92*	19.57	0.15	283.52	8.56**
SU30 × *S*	1	0.1	0.01	0.01	0	140.68	1.1	2.86	0.09
SU30 × *G*	1	11.25	1.02	33.43	1.58	0.09	0	4.58	0.14
R20 × *S*	1	10.61	0.96	6.12	0.29	8.73	0.07	34.29	1.04
R20 × *G*	1	144.82	13.09***	9.86	0.47	79.54	0.62	245.67	7.42**
SDII × *S*	1	15.10	1.37	3.75	0.18	50.36	0.39	31.55	0.95
SDII × *G*	1	25.67	2.32	68.72	3.25	87.84	0.69	59.37	1.79
BA × *S*	1	3.43	0.31	0.36	0.02	441.67	3.45	11.58	0.35
BA × *G*	1	13.6	1.23	72.84	3.45	99.06	0.77	267.75	8.08**
Residual	209	11.06		21.12		128.1		33.13	
Total	239								

(H1) species exclusive of grassland, pasture, rocky slope, rock; (H2) species of grassland, pasture and other habitat; (H3) species of scrubland and woodland; (H4) species of ex-arable, arable and urban areas (synanthropic species); (E) species endemic to Italian territory, (M) steno- Mediterranean species, (EBT) European, Boreal and Temperate species, (C) Cosmopolitan and Subcosmopolitan species.

* $P \leq .05$.
** $P \leq .01$.
*** $P \leq .001$.

underline that we are dealing with a small number of species (7 endemics), hence it is hard to say if we are observing a general trend. Grazing had a beneficial effect on the diversity of the restored communities which, without disturbance, form a poor community dominated by *B. erectus* and *P. spinosa* (Maccherini and Santi, 2012). In fact, in non-disturbed plots *B. erectus* forms a dense, tufted and perennial thick cover that impedes the colonization of any other species, included the synanthropic ones. The increase in *B. erectus* cover leads to a decrease in cover of many species present in other grasslands, such as in *biancana* badland in Tuscany (Maccherini et al., 2000). This result appears reversed when considering the interaction with climatic events: in grazed plots drought or heavy rain had a detrimental effect on restoration, increasing the cover of synanthropic undesirable species and decreasing the cover of more valuable endemic species. The analyses on the solely

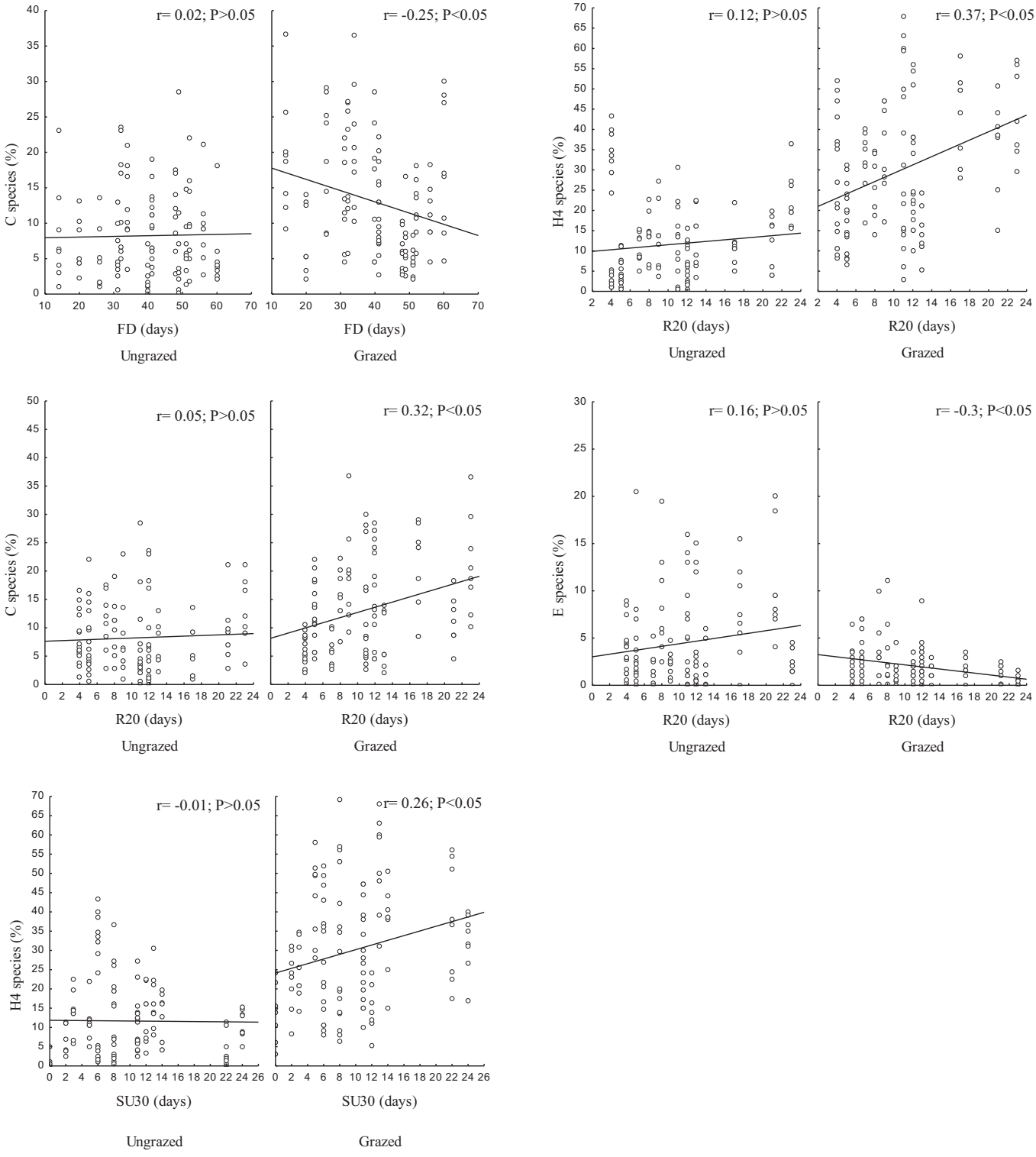


Fig. 4. The extreme climate indices correlations with percentage cover of species, shown separately for the two levels of the factor grazing. FD - Frost days; SU30 - Summer days; R20 - number of very heavy precipitation days; H4 - generalist species of arable and ex-arable (synanthrope species); E - endemic to Italian territory or part of this, C- Cosmopolitan and Subcosmopolitan species.

richness of species, showed a lower sensitivity to the average precipitation per wet day (SDII) of the richest grazed plots compared to those ungrazed; this would seem to confirm the generality of the consolidated equation, valid for moderate climate fluctuation, that the richest communities are the more resistant and suggest that it might not be proper dealing with climate extremes (Isbell et al., 2015; De Boeck et al., 2017).

Results confirm that total species richness per se is not a suitable indicator for restoration needs or targets and, in following the restoration outcomes, we should be very cautious in interpreting the change in total species richness values (Török and Helm, 2017). To shift towards a more ecological approach we must distinguish the contribution of the different species: target species, or the "characteristic diversity" species that are coherent with the composition of the reference community (e.g. H1); all the other species, the "derived diversity", which are generalists, alien species or simply plants that are characteristic of other non-target habitat types (e.g. H4), (Török and Helm, 2017; Helm et al., 2015; Maccherini et al., 2007).

Long-term data reveal complex dynamics in the studied grasslands, in relation to extreme climate and disturbance, as underlined by Hobbs et al. (2007). Still, a consistent portion of the variability between experimental units is attributable to unknown space–time variables: block and year of observation remain significant factors for almost all the response variables.

In a context of progressive tropicalization of the Mediterranean area, it is important to note that restoration actions should take into account the interactions existing between restoration treatments and climatic events: the effects caused by the disturbance (in our case, grazing) can be reversed by climatic extremes leading to a community that is different from the target one or, even worse, to a general biotic homogenization (McKinney and Lockwood, 1999). To cope with the rapid ecosystem change, conservationists and managers must deal with the novel, emerging or "no-analog" ecosystems (Hobbs et al., 2014) and everyone, involved in restoration, should take into consideration that the beneficial effects of restoration practices can be thwarted by climate change, when setting realistic restoration goals.

Author contributions statement

S.M. conceived the ideas and designed methodology; S.M. and G.B. collected the data; S.M. and G.B. analyzed the data; S. M., G. B. and M. M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

The authors thank Paolo Castagnini and Elisa Santi for help with sampling, the personnel of the Parco Faunistico del Monte Labbro for their technical support and Sula E. Vanderplank for revising the final text. This study was funded by the European Commission LIFE-Nature Project LIFE NAT/IT/99/6229 and LIFE04NAT/IT/000173 and by the Comunità Montana del Monte Amiata Zona "I1", Area Grossetana.

References

Anderson, M.J., 2001. Permutational test for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58:626–639. https://doi.org/10.1139/f01-004.
Anderson, M.J., ter Braak, C.J.F., 2003. Permutation tests for multifactorial analysis of variance. J. Stat. Comput. Simul. 73:85–113. https://doi.org/10.1080/00949650210000155 58.
Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.
Bakker, J.P., Berendse, F., 1999. Constraints in the restoration of ecological diversity in grassland and heathland communities. Trees 14, 63–68.
Benedetti-Cecchi, L., Osio, G.C., 2007. Replication and mitigation of effects of confounding variables in environmental impact assessment: effect of marinas on rocky-shore assemblages. Mar. Ecol. Prog. Ser. 334:21–35. https://doi.org/10.3354/meps334021.
Butaye, J., Dries, A., Honnay, O., 2005. Conservation and restoration of calcareous grasslands: a concise review of the effects of fragmentation and management on plant species. Biotechnol. Agron. Soc. Environ. 9, 111–118.
Chapman, M.G., Underwood, A.J., 2000. The need for a practical scientific protocol to measure successful restoration. Wetlands (Australia) 19, 28–48.
Chelli, S., Wellstein, C., Campetella, G., Canullo, R., Tonin, R., Zerbe, S., Gerdol, R., 2017. Climate change response of vegetation across climatic zones in Italy. Clim. Res. 71: 249–262. https://doi.org/10.3354/cr01443.
Chiarucci, A., Maccherini, S., 2007. Long-term effects of climate and phosphorus fertilisation on serpentine vegetation. Plant Soil 293:133–144. https://doi.org/10.1007/s11104-007-9216-6.
Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: USers Manual/Tutorial PRIMER-E: Plymouth, England.
Critchley, C.N.R., Burke, M.J.W., Stevens, D.P., 2004. Conservation of lowland semi-natural grasslands in the UK: a review of botanical monitoring results from agri-environment schemes. Biol. Conserv. 115:263–278. https://doi.org/10.1016/S0006-3207(03)00146-0.
De Boeck, H.J., Bloor, J.M.G., Kreyling, J., Ransijn, J.C.G., Nijs, I., Jentsch, A., Zeiter, M., 2017. Patterns and drivers of biodiversity–stability relationships under climate extremes. J. Ecol. 00:1–13. https://doi.org/10.1111/1365-2745.12897.
Dengler, J., Janišová, M., Török, P., Wellstein, C., 2014. Biodiversity of Palaearctic grasslands: a synthesis. Agric. Ecosyst. Environ. 182:1–14. https://doi.org/10.1016/j.agee.2013.12.015.
Di Castri, F., Hansen, A.J., Debussche, M., 1990. Biological Invasions in Europe and the Mediterranean Basin. Springer, p. 52 (ISBN 978-0-7923-0411-1).
van Dijk, G., 1991. The status of semi-natural grasslands in Europe. In: Goriup, P.D., Batten, L.A., Norton, J.A. (Eds.), The Conservation of Lowland Dry Grassland Birds in Europe. JNCC Publ., Newbury, pp. 15–36.
Dzwonko, Z., Loster, S., 2011. Impact of weather on dynamics of plant functional groups in an abandoned limestone grassland. Acta Soc. Bot. Pol. 80:259–268. https://doi.org/10.5586/asbp.2011.038.
Eskelinen, A., Harrison, S.P., 2015. Resource colimitation governs plant community responses to altered precipitation. Proc. Natl. Acad. Sci. U. S. A. 112:13009–13014. https://doi.org/10.1073/pnas.1508170112.
Frich, P., Alexander, L.V., Della-Marta, P., Gleason, B., Haylock, M., Klein Tank, A.M.G., Peterson, T., 2002. Observed coherent changes in climatic extremes during the second half of the twentieth century. Clim. Res. 19:193–212. https://doi.org/10.3354/cr019193.
Galvánek, D., Leps, J., 2009. How do management and restoration needs of mountain grasslands depend on moisture regime? Experimental study from north-western Slovakia (Western Carpathians). Appl. Veg. Sci. 12:273–282. https://doi.org/10.1111/j.1654-109X.2009.01022.x.
Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Glob. Planet. Chang. 63:90−104. https://doi.org/10.1016/j.gloplacha.2007.09.005.
Han, C., Young, S.L., 2014. Drought and grazing disturbances and resistance to invasion by warm- and cool-season perennial grassland communities. Ecol. Restor. 32:28–36. https://doi.org/10.3368/er.32.1.28.
Harris, J.A., Hobbs, R.J., Aronson, J., 2006. Ecological restoration and global climate change. Restor. Ecol. 14, 170–176.
Helm, A., Zobel, M., Moles, A.T., Szava-Kovats, R., Pärtel, M., 2015. Characteristic and derived diversity: implementing the species pool concept to quantify conservation condition of habitats. Divers. Distrib. 21:711–721. https://doi.org/10.1111/ddi.12285.
Hobbs, R.J., Yates, S., Mooney, H.A., 2007. Long-term data reveal complex dynamics in grassland in relation to climate and disturbace. Ecol. Monogr. 77, 545–568.
Hobbs, R.J., Higgs, E., Hall, C.M., Bridgewater, P., Chapin, F.S., Ellis, E.C., Ewel, J.J., Hallett, L.M., Harris, J., Hulvey, K.B., 2014. Managing the whole landscape: historical, hybrid, and novel ecosystems. Front. Ecol. Environ. 12:557–564. https://doi.org/10.1890/130300.
Hoover, D.L., Knapp, A.K., Smith, M.D., 2014. Resistance and resilience of a grassland ecosystem to climate extremes. Ecology 95:2646–2656. https://doi.org/10.1890/13-2186.1.
IPCC, 2007. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007. Working Group I: The Physical Sciences Basis. Contribution of Working Group 1 to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
ISAC-CNR, 2009. Clima, cambiamenti climatici globali e loro impatto sul territorio nazionale. Quaderni dell'ISAC. Vol 1. ISAC-CNR, Bologna.
Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., ... Eisenhauer, N., 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature 526:574–577. https://doi.org/10.1038/nature15374.
Jentsch, A., Kreyling, J., Elmer, M., Gellesch, E., Glaser, B., Grant, K., Hein, R., Lara, M., Mirzae, H., Nadler, S.E., Nagy, L., Otieno, D., Pritsch, K., Rascher, U., Schädler, M., Schloter, M., Singh, B.K., Stadler, J., Walter, J., Wellstein, C., Wöllecke, J., Beierkuhnlein, C., 2011. Climate extremes initiate ecosystem-regulating functions while maintaining productivity. J. Ecol. 99:689–702. https://doi.org/10.1111/j.1365-2745.2011.01817.x.
Lazzarotto, A., 1993. Elementi di geografia e geomorfologia. In: Giusti, F. (Ed.), La Storia naturale della Toscana meridionale. Monte dei Paschi di Siena, Cinisiello Balsamo (Milano), pp. 11–17.
Lloret, F., Escudero, A., Iriondo, J.M., Martínez-Vilalta, J., Valladares, F., 2012. Extreme climatic events and vegetation: the role of stabilizing processes. Glob. Chang. Biol. 18: 797–805. https://doi.org/10.1111/j.1365-2486.2011.02624.x.
Maalouf, J.-P., Le Bagousse-Pinguet, Y., Marchand, L., Bâchelier, E., Touzard, B., Michalet, R., 2012. Integrating climate change into calcareous grassland management. J. Appl. Ecol. 49:795–802. https://doi.org/10.1111/j.1365-2664.2012.02151.x.
Maccherini, S., Santi, E., 2012. Long-term experimental restoration in a calcareous grassland: identifying the most effective restoration strategies. Biol. Conserv. 146: 123–135. https://doi.org/10.1016/j.biocon.2011.11.032.

Maccherini, S., Chiarucci, A., De Dominicis, V., 2000. Structure and species diversity of *Bromus erectus* grasslands of biancana badlands. Belg. J. Bot. 133, 3–14.
Maccherini, S., Marignani, M., Castagnini, P., van den Brink, P.J., 2007. Multivariate analysis of the response of overgrown semi-natural calcareous grasslands to restorative shrub cutting. Basic Appl. Ecol. 8:332–342. https://doi.org/10.1016/j.baae.2006.06.002.
Maccherini, S., Santi, E., Marignani, M., 2014. Detection of the effects of restoration on community composition in a calcareous grassland: does scale matter? Grassl. Sci. 60:31–35. https://doi.org/10.1111/grs.12036.
McKinney, M.L., Lockwood, J.L., 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. Trends Ecol. Evol. 14, 450–453.
Moore, P.D., Chapman, S.B., 1986. Methods in plant ecology. Blackwell Scientific Publications, Oxford.
Pignatti, S., 1982. Flora d'Italia. Vol. I-III – Edagricole, Bologna.
Pistoi, M., 1989. Guida archeologica del Monte Amiata. Nuova Immagine Editrice, Siena, Siena.
Regione Toscana, 2012. P.A.E.R. Proposta di Piano Ambientale ed Energetico Regionale. Regione Toscana, Italy.
Ridding, L.E., Redhead, J.W., Pywell, R.F., 2015. Fate of semi-natural grassland in England between 1960 and 2013: a test of national conservation policy. Glob. Ecol. Conserv. 4:516–525. https://doi.org/10.1016/j.gecco.2015.10.004.
Smith, M.D., 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. J. Ecol. 99:656–663. https://doi.org/10.1111/j.1365-2745.2011.01798.x.
StatSoft Italia srl, 2001. STATISTICA per Windows. StatSoft Italia S.r.l, Padova.
Stevenson, M.J., Bullock, J.M., Lena, K.W., 1995. Re-creating semi-natural communities: effect of sowing rate on establishment of calcareous grassland. Restor. Ecol. 3, 279–289.
Stewart-Oaten, A., Murdoch, W.W., Parker, K.R., 1986. Environmental impact assessment: 'pseudoreplication' in time? Ecology 67, 929–940.
Stuble, K.L., Fick, S.E., Young, T.P., 2017. Every restoration is unique: testing year effects and site effects as drivers of initial restoration trajectories. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.12861 In press.
Suttle, K.B., Thomsen, M.A., 2007. Climate change and grassland restoration in California: lessons from six years of rainfall manipulation in a North Coast grassland. Madrono 54:225–233. https://doi.org/10.3120/0024-9637(2007)54[225:CCAGRI]2.0.CO;2.
Toreti, A., Desiato, F., 2008. Changes in temperature extremes over Italy in the last 44 years. Int. J. Climatol. 28:733–745. https://doi.org/10.1002/joc.1576.
Török, P., Helm, A., 2017. Ecological theory provides strong support for habitat restoration. Biol. Conserv. 206:85–91. https://doi.org/10.1016/j.biocon.2016.12.024.
Valéry, L., Fritz, H., Lefeuvre, J.-C., Simberloff, D., 2009. Invasive species can also be native…. Trends Ecol. Evol. 24:585. https://doi.org/10.1016/j.tree.2009.07.003.
Waldén, E., Lindborg, R., 2016. Long term positive effect of grassland restoration on plant diversity - success or not? PLoS One 11, e0155836. https://doi.org/10.1371/journal.pone.0155836.
Willems, J.H., 2001. Problems, approaches, and results in restoration of Dutch calcareous grasslands during the last 30 years. Restor. Ecol. 9:147–154. https://doi.org/10.1046/j.1526-100x.2001.009002147.x.
Wilson, J.B., Peet, R.K., Dengler, J., Pärtel, M., 2012. Plant species richness: the world records. J. Veg. Sci. 23:796–802. https://doi.org/10.1111/j.1654-1103.2012.01400.x.
Zhang, X., Alexander, L., Hegerl, G.C., Jones, P., Klein Tank, A., Peterson, T.C., Trewin, B., Zwiers, F.W., 2011. Indices for monitoring changes in extremes based on daily temperature and precipitation data. WIREs Clim. Change 2:851–870. https://doi.org/10.1002/wcc.147.